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PRINCIPAL INVESTIGATOR: Zhou Songyang, Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, Texas 77030-3498

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| Baylor College of Medicine Houston, Texas 77030-3498 E-Mail: songyang@bcm.tmc.edu | | U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012 | |
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| <p>Androgens and the androgen receptor (AR) play a critical role in the development and progression of prostate cancers. The majority of prostate cancers initially respond to endocrine treatment (androgen dependent), but eventually become androgen independent that prove fatal. It appears that a functionally active AR may contribute to the progression of androgen-independent prostate cancers. Understanding the signaling pathways that regulate androgen-dependent and -independent activation of AR mediated transcription would provide valuable information for finding an ultimate cure for this disease. We have proposed to identify the signaling components that regulate AR activity using a novel retrovirus-mediated genetic system, and to understand the mechanism of how the identified factors control AR activity. To this end, we have established and optimized our retrovirus-mediated genetic screen approach. The retroviral vectors to be used have been further modified and improved which should provide high-efficiency mutagenesis as well as ease of manipulation and analysis. We have also made significant progress towards generating a suitable cell line for the genetic screen. With these tools in hand, we will perform a genome wide genetic screen to identify genes that are important for androgen receptor signaling and study how they may contribute to the progression of prostate cancers.</p> | | | |
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Table of Contents

| | |
|--|----------|
| Cover..... | 1 |
| SF 298..... | 2 |
| Introduction..... | 4 |
| Body..... | 4 |
| Key Research Accomplishments..... | 6 |
| Reportable Outcomes..... | 6 |
| Conclusions..... | 6 |
| References..... | 6 |

Introduction

Androgens play a critical role in regulating prostate development and the evolution of prostate cancer (1). Androgens exert their biological function by activating gene transcription through the androgen receptor (AR), a member of the steroid nuclear receptor superfamily (2). Initially, endocrine treatment of prostate cancer patients either by inhibition of AR/androgen binding or deprivation of androgens can result in tumor regression. However, most prostate cancer patients relapse as the tumors become androgen independent (1, 3, 4, 5). An important step towards prostate cancer remedies is to understand the signaling pathways that regulate androgen-dependent and -independent activation of AR-mediated transcription. We propose to utilize a genetic system to screen for genes that regulate AR activity in cultured cells through high efficiency mutagenesis using Enhanced Retroviral Mutagens (ERM)(6). The overall objective of this proposal is to understand the molecular machinery that regulates AR transcription in normal and tumor prostate cells. We will first establish a genetic screening method to systematically search for genes that modulate AR activity *in vivo* using the PSA (prostate-specific antigen) enhancer and ARE as a reporter. How the identified genes regulate AR transcription activity and subcellular localization in the context of chromatin will then be investigated.

Body

For Task 1, we have proposed to isolate genes that are involved in regulating the activity of androgen receptor. To accomplish this, we proposed to generate a HeLa-AR cell line that would express luciferase and FKBP-caspase driven by ARE, and to use this cell line for genetic screens using engineered retrovirus mutagens. The rationale is to isolate clones of cells that can escape androgen-dependent caspase-mediated apoptosis as a result of retrovirus mediated mutagenesis. Subsequently, the gene loci that have been mutated to confer androgen independence in these clones can be identified.

We have generated a stable HeLa cell line that expresses the androgen receptor (pCMC-AR). This cell line was then used to introduce constructs that encoded luciferase, HSV thymidine kinase, and FKBP-caspase driven by ARE. These stable cells were then tested for their abilities to undergo apoptosis in the presence of androgen. We found that in the presence of androgen, less than 50% of cells underwent apoptosis. These cells can be used for the genetic screen. However, the high level of survival cells in the presence of apoptosis inducing signals will result in high background during the genetic screen. It will significantly increase not only the number of clones that have to be analyzed, but also the chance that authentic mutant clones would be missed. Therefore, we decided to generate a new cell line.

First, to circumvent the high background problem, we constructed new vectors. These new constructs contain 2xGRE upstream of GFP-NTR (Green fluorescent protein-nitroreductase) (7). Successful integration of this construct would allow the cells to express GFP upon androgen stimulation, and NTR allows drug selection against GFP-NTR expression. This construct was tested in both the stable AR-expressing HeLa cells and the LNCaP cells that contain AR. The expression constructs were first tested in transient transfection assays (Table 1). (Two separate constructs have been generated for expression in HeLa and LNCaP cells respectively).

Table 1. Percentage of GFP+ cells upon androgen (R1881) treatment in HeLa cells

| | -R1881 | +R1881 |
|----------------|--------|--------|
| pLCGGN + pCRAR | <1% | ~95% |
| pGGN + pCRAR | <1% | ~95% |

We concluded that the constructs were working as intended. We then went on to generate stable HeLa cells expressing both constructs. The cells were analyzed beforehand to ensure that no cells were expressing GFP before R1881 induction. And stable clones were then selected using G418. We have already obtained stable clones. These clones were first tested for their ability to undergo apoptosis in the presence of metronidazole (MN) (Table 2).

Table 2. Response of HeLa cells stably expressing NTR to metronidazole

| MN (mM) | 0.1 | 0.5 | 1 | 5 | 10 | 15 |
|-----------|-----|-----|---|---|----|----|
| apoptosis | - | - | - | - | + | + |

We then determined that the majority of the stable cells expressed GFP in response to R1881, similar to the data obtained from the transient transfection experiment (Table 1). Finally, two populations of cells were selected to obtain GFP positive or negative cells that were resistant to MN treatment. Both types of cells will be used in the next step for the ERM-mediated genetic screen. The following flow chart illustrates the selection method (Figure 1). We are currently in the process of finalizing the conditions for the genetic screen using these cells.

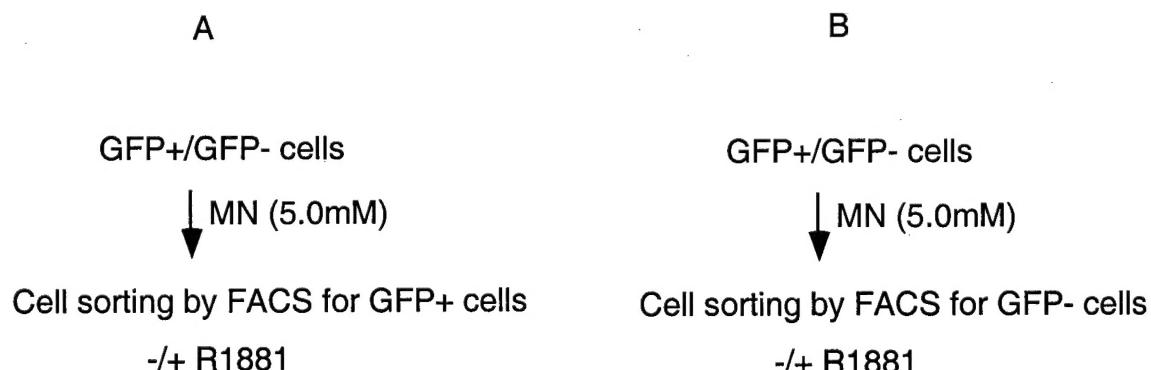


Figure 1. Flow chart for selection of stable cells resistant to MN

In the mean time, we have also optimized our ERM vectors to be used in the genetic screen. We have modified the epitope tags and promoter regions to improve the mutagenesis efficiency and ease of manipulation. Because Task 2 is dependent on Task 1, we expect to make significant and new progress once Task 1 is completed.

Key Research Accomplishments

- Establishment and improvement of the ERM genetic screen approach
- Generation of the androgen-responsive reporter cell line for genetic screen

Reportable Outcomes

We have engineered improved versions of the retrovirus based genetic screen vectors. These new ERM vectors should provide high efficiency mutagenesis. Furthermore, they should provide easier makers and epitopes for analysis and manipulation.

At the same time, we have obtained the cell lines that are more suitable for genome wide genetic screens using ERM vectors.

Conclusions

In summary, we have further improved our genetic screen approach for high efficiency mutagenesis. Although our progress was somewhat hampered due to difficulty in generating a suitable cell line to use for the genetic screen, we have overcome this difficulty by utilizing different markers. We are well on our way to starting the genetic screen and obtaining mutant clones. The genes that have been mutated in these clones will then be identified and studied to examine how they may affect the androgen receptor signaling pathway. Such information will prove invaluable in our quest for the cure of prostate cancers.

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